Special Techniques: Proposed protocols

Removing Magnetic Materials

1. After sample concentration, transfer the pellet into a Leighton tube without Buffers A and B
2. Place the Leighton tube in the MPC®-1 or MPC®-6
3. Check the flat side of the tube is flat and tight against the magnet
4. Rock the magnet and tube through a 90° angle
5. Rock gently and smoothly for 2 minutes, ~1 second for each 90° rock
    a. Ensure the tilting action is continued throughout the 2 minute period
    b. If the sample is allowed to stand motionless for more than 10 seconds:
        i. remove the tube from the magnet, shake to resuspend all materials
        ii. replace the sample tube in the magnet, repeat the 2 minute rocking
6. Return the tube to upright position and immediately remove the cap
7. Keep flat side of tube on top:
    a. pour off supernatant into a second Leighton tube which contains Buffers A and B
    b. this portion of the sample will continue through the IMS process
8. Add reagent water to the second Leighton tube to bring the total volume to 12 mL
9. Continue sample processing with step 13.3.2.1 of Method 1623, 2005 version to completion of method.

Notes:

- The first Leighton tube should contain extraneous iron and other magnetic material removed by the magnet.

Reference:

Appl Environ Microbiol. 2002 April; 68(4): 2066–2070. doi: 10.1128/AEM.68.4.2066-2070.2002.

Effects of pH and Magnetic Material on Immunomagnetic Separation of *Cryptosporidium* Oocysts from Concentrated Water Samples
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Special Techniques: Proposed protocols

Adjusting pH

1. Process the sample through step 13.3.2.1 as stated in the Method.
2. Gently mix the buffers with the transferred sample by inverting the Leighton tube 3 times
3. Record the pH of suspension
4. Adjust the pH of the suspension with 1N HCL or 1N NaOH as needed to establish pH = 7
5. Continue sample processing with step 13.3.2.2 of Method 1623, 2005 version to completion of method.

EQUIPMENT:

Use IQ Scientific Instruments for non-glass pH probes and micro probes, to check pH in the Leighton tube.

Notes:

- pH readings of the sample may also be collected in the centrifuge tube prior to transfer to the Leighton tube and in the Leighton tube after rotation to confirm stability of the pH.

Reference:

Appl Environ Microbiol. 2002 April; 68(4): 2066–2070. doi: 10.1128/AEM.68.4.2066-2070.2002.

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Special Techniques: Proposed protocols

Heat Dissociation

1. Process sample(s) according to Method 1623, 2005 version, through Section 13.3.3.1
2. Remove the microcentrifuge tube from magnetic strip of the MPC®-S
3. Add 50 µL of reagent water, vortex at highest setting for approximately 50 seconds
4. Place tube(s) in 80ºC heat block for 10 minutes
5. Remove tube(s) from heat block, vortex at highest setting for approximately 30 seconds
6. Ensure all of the sample is at the base of the tube, then place the tube in the MPC®-S with the magnet in the slanted position
7. Allow tube to stand undisturbed for a minimum 10 minutes
8. Prepare a well slide for sample examination and label the slide
9. Without removing the microcentrifuge tube from the MPC®-S, transfer all of the sample from the microcentrifuge tube to the well slide
   Do not disturb the beads at the back wall of the tube; ensure all of the fluid is transferred.
10. Do not discard the beads or microcentrifuge tube after transferring the volume from the first heat dissociation to the well slide. Perform steps 2-9 again.
11. The volume from the second heat dissociation can be added to the slide containing the volume from the first dissociation, or can be applied to a second slide.
12. Continue sample processing with step 13.3.3.11 of Method 1623, 2005 version to completion of method.

EQUIPMENT:

Use a Multi Block Heater, Model 2050 or Grant UBD1 heat block [The Grant UBD1 heat block has options of various block sizes to accommodate different plasticware including a 1.5 mL microtube interchangeable block BB-E1,that can hold 24 tubes at once.]; or equivalent

Reference:

http://oaspub.epa.gov/eims/xmlreport.display?deid=63880&z_chk=3407
Ware, M. W., L. Wymer, H. A. Lindquist, AND F. W. Schaefer III. EVALUATION OF CRYPTOSPORIDIUM OOCYST RECOVERY IN WATER BY EPA METHOD 1623 WITH A MODIFIED IMS DISSOCIATION PROCEDURE. Presented at International Symposium on Waterborne Pathogens, Lisbon, Portugal, September 22-25, 2002.